Aquatic Botany 148 (2018) 46–52

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Short-term interactive effects of increased temperatures and acidification on the calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis*

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ARTICLE INFO

Keywords:
Climate change
Acidification
Warming
Macroalgae

ABSTRACT

Combined effect of acidification and sea warming derived from future conditions of Climate Change have been little investigated in marine photoautotrophs, especially on sensitive organisms such as calcifying macroalgae. The aim of this investigation was to evaluate the interactive effects of acidification and increased temperatures on the two Brazilian calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis*. Both species were cultured for 48 h under: **(1)** current pH (7.8 ± 0.2) and temperature (18 ± 2 °C) during winter; **(2)** future pH (7.4 ± 0.2) and temperature (30 ± 2 °C) during summer; **(3)** current temperature and future pH; and **(4)** future temperature and current pH. We evaluated photosynthetic performance (measured $\Delta F/F'_m$), growth rates (weight), relative carbonate content, and total phenolic compounds. Our results showed similar negative effects under decreased pH and increased temperatures in both species, although carbonate content *S. capensis* was less affected than in *L. crispatum*. Total phenolic compounds measured in *S. capensis* showed the highest levels at potential future pH and temperature conditions. Given that stress conditions associated with decreased pH and increased temperatures are important inductors of an oxidative response, it is likely that phenolic compounds are synthetized to fulfil an antioxidant purpose. Even though physiological performance was affected in both calcifying macroalgae under the most likely negative future pH and temperature conditions, their biological viability indicates they may be able to thrive under coming Climate Change scenarios.

1. Introduction

Atmospheric carbon dioxide (CO_2) concentrations have been increasing from 280 ppm since the pre-industrial period to above 400 pm nowadays; moreover, CO_2 levels are expected to increase in up to 970 ppm by the end of the 21st Century (Harley et al., 2006, 2012). Predicted CO_2 rise and accompanied increase in global temperatures is expected to induce greater sea surface temperatures, which could fluctuate between 2 and 6 °C depending on the strictness of the extrapolation (IPCC, 2014). On the other hand, approximately 25% of atmospheric CO_2 is absorbed by the ocean, which in reaction with seawater increases the levels of carbonic acid (H_2CO_3), subsequently diminishing the pH; this process is expected to induce a 0.2–0.4 pH decline by the year 2100 (Feely et al., 2004). Ocean acidification produces changes in total dissolved inorganic carbon (DIC), manifested in higher concentration of carbonate ions (CO_3^{2-}) and bicarbonate (HCO_3-); important molecules that may affect physiological processes, mostly in marine calcifying organisms (Martin and Hall-Spencer, 2017; Orr et al., 2005; Koch et al., 2013). As signal of Climate Change, monitoring of coastal marine habitats has revealed a concerning increase in the incidence of mass mortalities in flora and fauna due to marine heat waves as observed in Australia (Wernberg et al., 2011) or in the south Atlantic (Ferreira et al., 2015). Elevated temperature, even over a short period, can be lethal due to biochemical damages (Gouvêa

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https://doi.org/10.1016/j.aquabot.2018.04.008
Received 22 December 2017; Received in revised form 16 March 2018; Accepted 21 April 2018
Available online 26 April 2018
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et al., 2017). In these coastal and shallow regions, high pH variability is observed, eventually exposing organisms to conditions frequently predicted to the end of this century considering ocean acidification (Duarte et al., 2013). Changes in ocean circulation also provide localized and short variability in ocean pH due upwelling/downwelling zones (Gruber et al., 2012; Findlay et al., 2013), CO_2 enrichment from volcanic vents (Hall-Spencer et al., 2008), and as a function of the metabolism of some marine communities (Anthony et al., 2011) or even in anoxic zones (Melzner et al., 2013). Therefore, extreme values of temperature and pH/pCO_2 present in different regions of the world can accentuate or even represent nowadays ocean acidification impacts on the biology of marine biodiversity.

Calcifying macroalgae are key ecological components of the Atlantic coast. They are considered ecosystem bioengineers, as create habitat, niche for settlement and nursery for other algae, invertebrates, and fish (Figueiredo et al., 2012; Riul et al., 2009; Amado-Filho et al., 2007). Furthermore, these organisms are major producers of carbonate sediment in the marine environment (Basso, 2012). Previous studies have shown that unbalanced DIC mediated by decreased pH reduces the saturation of calcium carbonate ($CaCO_3$) in calcifying marine organisms, such calcifying macroalgae, corals, foraminifera and coccolithophores; this process can affect calcification rates when producing new skeleton (Basso, 2012; Martin and Hall-Spencer, 2017; Orr et al., 2005). $CaCO_3$ occurs in skeletons of calcifying macroalgae as calcite or aragonite; however, magnesium (Mg^{2+}) can replace calcium in calcite macroalgae, which has been observed to be more prone to dissolution under acidification than macroalgae with aragonite skeleton (Basso, 2012; Jury et al., 2010; Smith et al., 2012).

Calcifying macroalgae are capable of performing calcification and photosynthesis at different rates depending on CO_2 concentrations (Hofmann et al., 2012; Hurd et al., 2009; Koch et al., 2013). Despite that acidification can produce adverse effects on calcification rates, changing carbonate content, high concentrations of CO_2 have been detected to favor photosynthesis in some cases (Hurd et al., 2009; Koch et al., 2013). For instance, Semesi et al. (2009) showed that a progressive decline in pH induced a 13% increase in photosynthesis rates in coralline algae, although compromising their structure due to 20% less calcification. Tropical and subtropical organisms survive near suboptimal physiological temperature; however, Climate Change-mediated increase in sea temperatures threatens their thermal tolerance thresholds (Latham, 2008; Eggert, 2012; Harley et al., 2006). Within tolerance ranges, rising temperature can induce an increase in photosynthesis, growth and carbonate deposition in calcifying macroalgae, but when these exceed tolerance thresholds, it can cause bleaching and chlorophyll degradation (Martin and Hall-Spencer, 2017; Glynn, 1996).

Drastic environmental disturbances can generate biological stress in macroalgae through the over-production of reactive oxygen species (ROS) (Bischof and Rautenberger, 2012). ROS excess can produce damage by oxidizing lipids, proteins and nucleic acids; however, the cell can maintain ROS within homeostatic levels through the production of antioxidant compounds (Moenne et al., 2016). In this context, phenolic compounds have been observed to have strong ROS scavenging properties (Sáez et al., 2015; Flores-Molina et al., 2016). ROS-induced increase in phenolic compounds in macroalgae have been observed subject to several environmental stressors, among which can be mentioned herbivory, changes in temperature, salinity, irradiance and metal excess (Bischof and Rautenberger, 2012; Moenne et al., 2016; Sáez et al., 2015; Flores-Molina et al., 2016).

Records on the interaction of different stressors associated with Climate Change on marine calcifying macroalgae are scarce; thus, more information on aspect such as the combined effects of acidification and increased temperatures would significantly improve our understanding on their current and future responses to progressive Climate Change. The aim of this research was evaluate the interactive effects of a short acidification and warming on physiological aspects of the calcifying red coralline *Lithothamnion crispatum* Hauck and the red aragonite *Sonderophycus capensis* (M) M.J. Wynne. *L. L. crispatum* has a wide range of latitudinal distribution; considered as a dominant species in the entire Atlantic coast of Brazil, its temperature tolerance ranges between 15–30 °C (Pascelli et al., 2013; Riul et al., 2009). On the other hand, *S. capensis* has a limited distribution (southeastern Atlantic of Brazil) with tolerance ranges defined between 17 and 20 °C (Zavialov et al., 1999). Taking into account limits of temperature tolerance and $CaCO_3$ types of skeleton in these species, it is expected that *L. crispatum* and *S. capensis* have differential responses to single and combined effects of increased temperatures and acidification.

2. Materials and methods

2.1. Collection and experimental design

Entire individuals of two calcifying red macroalgae species, the coralline *L. crispatum* and the aragonite-based *S. capensis,* were collected at 10 m depth from Reserva Biologica do Arvoredo, in Santa Catarina (27° 15′ S, 48° 20′ W) and in Xavier Island (27° 36′ S, 48° 23′ O), respectively, in Brazil. After collection, individuals were stored in plastic bags with seawater, and immediately transported inside coolers to the Laboratory of Phycology at Universidad de Santa Catarina. Prior to experiments, the specimens were acclimated for 24 h with filtered seawater (0.45 µm) under constant aeration, temperature (15 ± 2 °C) and light (50 µmol photons $m^{-2} s^{-1}$). The experiment was carried out in a microcosm system over a short-term period (48 h). The experimental system consisted in two tanks with 50 L seawater that delivered seawater with a pump to 12 chambers of 1 L. Both macroalgae species were randomly distributed and maintained in four conditions: **(1)** average current pH (7.8 ± 0.2) and temperature (18 ± 2 °C) registered in Florianopolis bay, Brazil, during winter **(2);** future values of pH (7.4 ± 0.2) and temperature (30 ± 2 °C) that can be extrapolated for Florianopolis bay during summer by the end of the 21st Century according to predictions by IPCC (2014); **(3)** Current temperature (18 ± 2 °C) and future pH (7.4 ± 0.2); and **(4)** Future temperature (30 ± 2 °C) and current pH (7.8 ± 0.2). For each treatment, three independent replicates were used. pH levels considered for control treatments respond to local levels registered in seawater nearby macroalgae collection sites. Certainly, in these locations there are several estuaries, such as Barra de Lagoa, that supply freshwater and inland material that induces a pH drop in coastal waters; in this context, several reports in this area show average pH levels around 7.8 (Martins-Pereira, 2004; Dalinghaus, 2016; Cancellier-Cechinel, 2013). Considering the latter, acidification treatments with a 0.4 pH decrease were made according IPCC predictions upon current local pH levels.

To achieve low pH conditions, bubbles of CO_2 were injected with a pump the experimental seawater. The added CO_2 were managed by a pH controller, model pH2010 (Weipro, China), connected to solenoid valves that regulated the amount of gas pumped into the seawater (Russell et al., 2009). Experimental temperatures were achieved by placing tanks on a homemade temperature-gradient table; this is a horizontal metal-made table, which surface temperature gradually increases from 15 °C to 35 °C, from one extreme to the other. In addition, the table constantly agitates to maintain temperature homogeneity in the seawater.

2.2. Water carbonate chemistry parameters

HCO_3^{-2} and CO_3^{-2} concentrations, pCO_2, calcite and aragonite saturation (Ω), were obtained via CO_2Sys_v2.1xls program, using K1, K2, as in Millero et al. (1998). These values are summarized in Table 1. Estimations were based in measurements of pH, temperature, salinity and total alkalinity in seawater. Gran titration method was used to measure total alkalinity, using 0.0025 N hydrochloric acid (HCl; Merck) (Carmouze, 1994); these measurements were taken after 48 h of experiments.

Table 1
Summary of the chemical characteristics of experimental seawater, including T°, pH, pCO_2, HCO_3^- and CO_3^{2-} concentration, $\Omega_{calcite}$ (Ω Ca) and $\Omega_{aragonite}$ (Ω Ar).

T(°C)	pH	pCO_2 (ppm)	HCO_3^- (µmol kg SW^{-1})	CO_3^{2-} (µmol kg SW^{-1})	ΩCa	ΩAr
18 ± 2	7.8 ± 0	432 ± 14	1164 ± 51	62.563 ± 3.2	1.491 ± 0.07	0.96 ± 0.04
18 ± 2	7.4 ± 2	2035 ± 19	1387 ± 13	18.816 ± 1.8	0.449 ± 0.04	0.290 ± 0.02
30 ± 2	7.4 ± 2	2504 ± 23	1590 ± 15	33.393 ± 3	0.811 ± 0.07	0.543 ± 0.05
30 ± 2	7.8 ± 0	455 ± 1.10	1148 ± 0.24	95.524 ± 0.6	2.319 ± 0.01	1.553 ± 0.01

Note: $n = 3$ (means ± SDs). Abbreviations: T (°C) = Temperature, pCO_2 = partial pressure of carbon dioxide, HCO_3^- = bicarbonate, CO_3^{2-} = carbonate, Ω Ca = Calcite saturation, Ω Ar = Aragonite Saturation.

2.3. Photosynthetic performance

Photosynthetic measurements were performed throughout the course of the experiments in all treatments, at 6, 24 and 48 h. Effective quantum yield of photosystem II (PII; $\Delta F/F'_m$) was measured using a portable pulse-amplitude modulated (PAM) chlorophyll α fluorometer (DIVING PAM; Walz GmbH, Effeltrich, Germany). This parameter was measured according to Schreiber and Neubauer (Schreiber and Neubauer, 1990): $\Delta F/F_m' = (F_m' - F_t)/F_m'$. In the latter, F_m' is the maximal fluorescence of light-acclimated sample induced by a saturating actinic light pulse (9000 µmol photons $m^{-2} s^{-1}$, 800 ms), and F_t is the intrinsic steady-state fluorescence emission in light-acclimated sample (Baker, 2008).

2.4. Growth rate

To estimate the growth, the change in fresh weight (FW) biomass of the species was measured, prior and after 48 h of experiments. The mean relative growth was expressed as FW per day (FW d^{-1}), and calculated according to Packard and Boardman (1999): $RG = W_t - W_0/t$. W_0 is the initial weight, W_t is final weight, and t is time in days (d).

2.5. Relative carbonate content (RCC)

Biomass was dried at 60 °C for 24 h and weighted. Initial weight (DWi) was obtained and then immersed in 10% HCl (Merck) for 24 h to initiate CaCO3 dissolution, in order to maintain the same weight/volume ratio. After the experiments, samples were dried (60 °C) again and were re-weighted (DWf) (Figueroa et al., 2014). RCC as a proxy of calcification was calculated to the formula: % RCC ((DWi-DWf)/DWf)*100).

2.6. Content of total phenolic compound

Due to biomass availability, total phenolic compounds were only determined in *S. capensis,* according to Randhir et al. (2002). After 48 h experiments, samples were immediately frozen in liquid nitrogen and stored at −80 °C. Between 40–50 mg biomass were grounded to powder in a mortar with liquid nitrogen, at 4 °C chamber in the darkness. One mL of 80% methanol (Merck) and 5 ml of 95% methanol (Merck) were added to each sample in centrifuge tubes. Samples were centrifuged at 10,000 rpm for 5 min at room temperature. One mL of the supernatant was placed in a clean tube and mixed with 5 mL of distilled water, 1 mL of 95% ethanol and 500 µL of Folin-Ciocalteu; the mixture was incubated for 5 min in the darkness. Then, 1 mL of $NaCO_3$ (5% w/v) was added and incubated in the dark for 1 h at room temperature. Finally, absorbance was measured in a spectrophotometer, model P-220 (Biospectro, Brazil), at 725 nm. To calculate the content of total phenolic compounds from crude extracts, a standard curve of known concentrations of gallic acid was used (50 to 800 µg mL^{-1}–$r^2 = 0.99$; y = 1.254).

2.7. Statistical analyses

Effects of temperature and pH on both species ($\Delta F/Fm'$, RCC, growth rate and phenolics content), and chemical changes in seawater, were assessed statistically by using a factorial analysis of variance (Factorial-ANOVA). A *post hoc* analysis of Student Newman-Keuls tests (SNK) was applied when Cochran's C test and visual inspection of residuals were conducted to test homogeneity of variances (Underwood, 1997). A level of significance of 99% confidence interval ($p = 0.01$) was applied. These analyses were performed using the software Statistica version 7 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Photosynthetic responses

For both species, a significant interaction was found between temperature (18 and 30 °C) and pH (7.8 and 7.4) on photosynthetic performance (measured as Δ *F/Fm'*) (Factorial ANOVA, $p < 0.01$; Fig. 1; Table 2). Δ *F/Fm'* significantly increased with respect to basal values in *L. crispatum* after 6, 24 and 48 h experiments at 18 °C under both 7.4 and 7.8 pH conditions (Fig. 1A). A similar trend was observed in *L. crispatum* at 30 °C under pH 7.4, although without significant differences at 24 h experiments (Fig. 1A). Δ *F/Fm'* significantly decreased under pH 7.8 at 30 °C in all experimental times, with lower values at 24 and 48 h experiments. In relation to *S. capensis*, no specific trends compared to basal values were observed at 18 °C under both pH conditions and at 30 °C under pH 7.4 (Fig. 1B). However, significantly

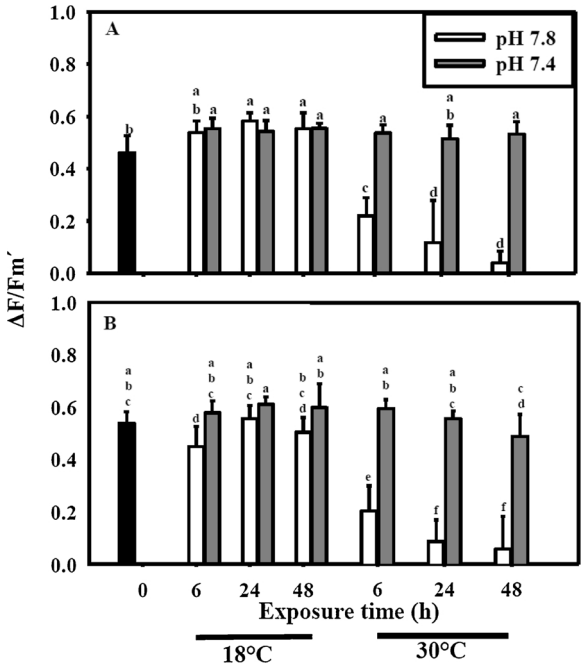

Fig. 1. Changes in maximum fluorescence *(Δ F/Fm')* emitted by **(A)** *Lithothamnium crispatum* and **(B)** *Sonderophycus capensis* exposed to increased temperatures and decreased pH for up to 48 h. Data correspond to mean ± SD ($n = 9$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p < 0.05$).

Table 2
Summary table of Factorial ANOVA of the effects of temperature and pH treatment on ΔF/Fm´ and calcification percentage in *Lithothamnion crispatum* and *Sonderophycus capensis*.

	Lithothamnion crispatum				*Sonderophycus capensis*			
	df	MS	F	*p*	*df*	MS	F	*p*
Temperature (T)	1	0.25	110.53	p < 0.01	1	0.11	25.940	p < 0.01
pH	1	0.25	108.05	p < 0.01	1	0.60	131.84	p < 0.01
T*pH	1	0.20	88.84	p < 0.01	1	0.15	33.76	p < 0.01
Error	32	0.002			32	0.004		
T	1	13.84	0.24	0.63	1	0.49	0.01	0.911
pH	1	4793.10	86.23	p < 0.01	1	5159.99	141.58	p < 0.01
T*pH	1	3301.41	59.40	p < 0.01	1	1474.34	40.45	p < 0.01
Error	8	55.58			8	36.45		

Note: $n = 8$ for ΔF/Fm´; $n = 3$ for calcification percentage. Abbreviations: T = Temperature, *df* = degrees of freedom.

lower Δ *F/Fm'* levels were recorded at 30 °C under pH 7.8, especially at 24 and 48 h experiments (Fig. 1; Table 2).

3.2. Responses in calcification

For both species a significant interaction was observed between temperature (18 and 30 °C) and pH (7.8 and 7.4) on RCC (Factorial ANOVA, $p < 0.01$; Fig. 2; Table 2). Similar trends were observed in RCC between studied species. When decreasing the pH from 7.8 to 7.4, RCC decreased significantly under both 18 and 30 °C (Fig. 2). The exception was at 30 °C in *L. crispatum*, where no significant differences were detected between both experimental pH conditions (Fig. 2).

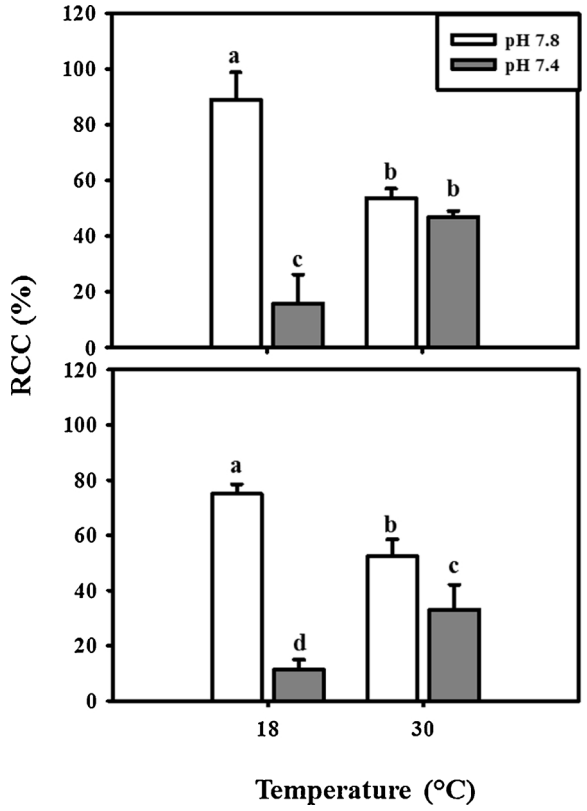

Fig. 2. Changes in relative carbon content (RCC), as a proxy of calcification rates, observed in **(A)** *Lithothamnium crispatum* and **(B)** *Sonderophycus capensis* exposed to increased temperature and decreased pH for up to 48 h. Data correspond to mean ± SD ($n = 3$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p < 0.05$).

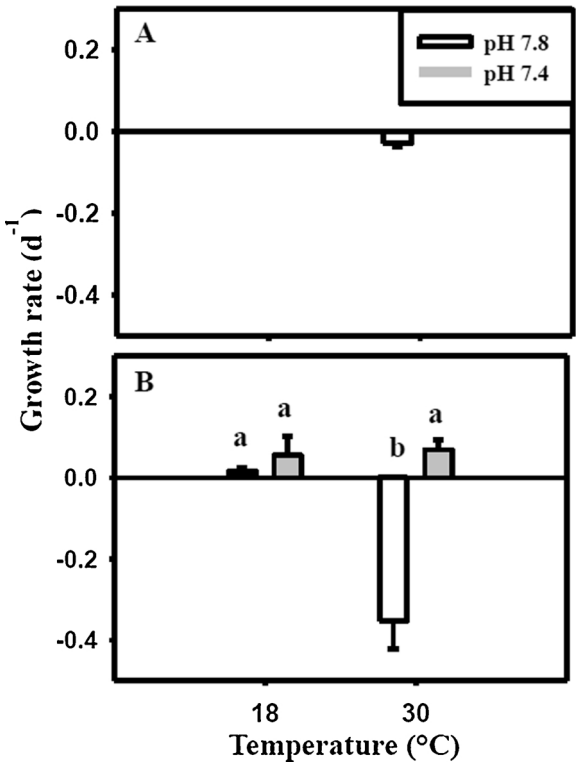

Fig. 3. Change in growth rate measured in **(A)** *Lithothamnium crispatum* and **(B)** *Sonderophycus capensis* exposed to increased temperature and decreased for up to 48 h. Data correspond to mean ± SD ($n = 3$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p < 0.05$).

3.3. Relative growth rate

No changes were recorded in growth rates in *L. crispatum,* with exception of the treatment at 30 °C and pH 7.8, where negative values were observed (Fig. 3A). A similar trend of negative growth rates were detected in *S. capensis* at 30 °C and pH 7.8, although the rest of the treatments were similarly positive (Factorial ANOVA, $p < 0.01$; Fig. 3B; Table 2).

3.4. Phenolic compound

A significantly interaction was observed between temperature (18 and 30 °C) and pH (7.8 and 7.4) on total phenolic content in *S. capensis* (Factorial ANOVA, p < 0.01; Fig. 4A). Under both experimental temperatures, total phenolic content increased at pH 7.4 with respect to 7.8.

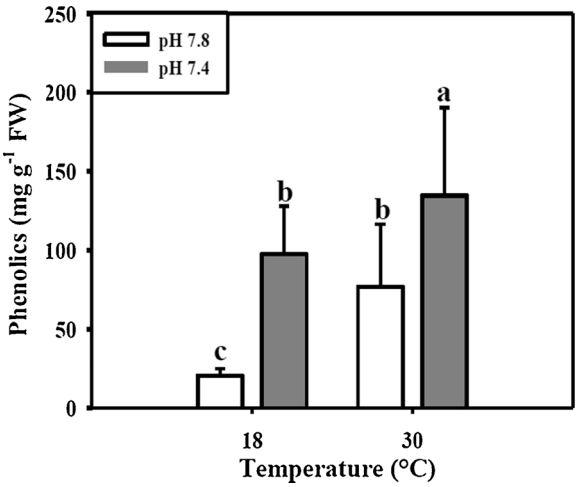

Fig. 4. Change in total phenolic compound in *Sonderophycus capensis* exposed to increased temperature and decreased pH for up 48 h. Data correspond to means ± SD ($n = 6$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p < 0.05$).

4. Discussion

In this investigation, it was observed that the two calcareous macroalgae *L. crispatum* and *S. capensis* suffered similar negative effects subject to the effects of increased temperatures, from 18 (similar to nowadays winter temperatures) to 30 °C (comparable to summer end of the 21st Century temperatures), or decreased pH, from current 7.8 to expected future 7.4 (by the year 2100); this was evidenced in terms of photosynthetic performance (Δ*F/Fm´*), relative carbon content (RCC) and growth rates. However, when increased temperatures (30 °C) were combined with acidification (pH 7.4), at least in terms of photosynthesis and growth, both species seemed to be less affected than when both stressors were applied independently. In terms of calcification, acidification at 18 °C caused a dramatic decay in RCC in both *L. crispatum* and *S. capensis*. Moreover, although RCC decreased under increased temperatures and acidification, compared to 18 °C and pH 7.8, levels remained always higher than at 18 °C and 7.4 pH; this for both calcareous macroalgae species. Indeed, increased temperatures appeared to diminish the negative effects induced by acidification, especially in relation to calcification, since at increased temperatures of 30 °C and low pH of 7.4 both macroalgae species displayed higher RCC if compared to treatments at 18 °C and pH 7.4. Total phenolic content measured in *S. capensis* showed the lowest levels at nowadays temperatures and pH. These increased similarly at 18 °C and pH 7.4, and at 30 °C and pH 7.8, and with the highest values at 30 °C and pH 7.4.

The detrimental effects of increased temperatures on *L. crispatum* and *S. capensis* could be attributed exceeding the tolerance thresholds of both species. Certainly, it has been observed that increased temperatures beyond tolerance ranges can induce an excess in the levels of reactive oxygen species (ROS) and subsequently oxidative stress in other photoautotrophs. For instance, it has been observed that the reaction catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), located in the chloroplast and involved in carbon fixation and photorespiration, can cause greater levels of the ROS H_2O_2 under high temperatures due to increased oxygenase reactions (Kim and Portis, 2004). Moreover, it has been observed that increased temperatures induce incomplete H_2O oxidation in the PSII electron donor side, forming H_2O_2, which is then reduced by manganese to the highly oxidizing HO· through Fenton reaction (Pospíšil, 2016). In this context, Müller et al. (2012) found a systematic increase in the ROS $\cdot O^{2-}$ in parallel with greater temperatures of up to 18 °C in the gametophytes of the brown macroalgae *Alaria esculenta*, *Laminaria digitata*, and *Saccharina latissima*. An extended oxidative stress in macroalgae is capable of causing damage and degradation to photosynthetic pigments; thus, affecting photosynthesis and development (Moenne et al., 2016). The effects of high temperatures have been reported in the rhodolith *Lithophylum margaritae*, which from 10 to 30 °C showed optimum oxygenic photosynthetic threshold at 25 °C; however, when values exceeded these temperatures photosynthesis began to decline (Steller et al., 2007). Reports by Vásquez-Elizondo and Enríquez (2016) demonstrated that from 30 °C to 32 °C, photosynthesis (F_v/F_m) suffered nearly a 45% decline after 3 d of experiments in rhodolith *Neogoniolithon* sp., articulate *Amphiroa tribulus* and crustuse *Lithothamnion* sp. Despite the latter, similar increased-temperature experiments (from optimal) on other tropical calcareous macroalgae, such as *Tricleocarpa cylindrica,Padina gymnospora, and Lithothamnion corallioides* have not evidenced negative effects in terms of photosynthetic performance (Scherner et al., 2016; Noisette et al., 2013). Therefore, photosynthetic performance under increased temperatures seems to be a species-specific feature in calcifying macroalgae.

In relation to the effects of acidification, our results demonstrated that negative effects of increasing temperatures from 18 to 30 °C were softened when decreasing the pH from 7.8 to 7.4. Our records are similar to those reported by Semesi et al. (2009), which observed that a progressive decrease in pH (from 9.8 to 7.6) were accompanied by an exponential increase in photosynthetic O_2 exchange in the rodolith *Hydrolithon* sp. Recent studies by Scherner et al. (2016) demonstrated the differential effects of low pH on photosynthesis in calcifying macroalgae; while from pH 8.1 to 7.2 F_v/F_m decreased in *Lithophyllum stictaeforme*, *Pneophyllum conicum* and *Porolithon pachydermum,* in *Tricleocarpa cylindrica* it increased. Additionally, Kram et al. (2015) observed no change in photosynthesis in the articulated *Jania adhaerens* and in the incrusting *Lithothamnion californicum* from pH 8.1 to 7.6, although growth rates decreased. Even though photosynthesis enhancement has been observed in different macroalgae species subject to acidification, published research suggests that this phenomenon may be more related to CO_2 availability rather than reduction of pH itself. According to Martin and Hall-Spencer (2017), higher concentration of CO_2 in seawater induces macroalgae to rely exclusively of CO_2 diffusion, own-regulating their CO_2-concentrating mechanisms (CCM) to save energy. Certainly, high availability of diffused CO_2 may provide greater substrate for RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbon fixation, inducing photosynthesis and growth (Martin and Hall-Spencer (2017).

The decalcification of calcifying macroalgae induced by acidification has been already well described (Basso, 2012; Feely et al., 2004). Likewise, we observed similar decalcification (about 80%) under lower pH from 7.8 to 7.4 at 18 °C in both *L. crispatum* and *S. capensis*. Semesi et al. (2009) showed that a progressive decrease in pH values from a 10 to 7.5 led to nearly 100% decalcification in *Hydrolithon* sp. Similarly, Noisette et al. (2013) observed decrease in calcification rates from pH 8.1 to 7.7 in *Lithothamnion corallioides*. Interestingly, these authors observed that at an increase in environmental temperatures reduced the impact of pH 7.7 in calcification rates. In this context, other studies on coralline algae have shown higher levels of calcification during summer, when average sea surface temperatures are the highest (Steller et al., 2007; Martin and Gattuso, 2009). Despite the latter, there are also investigations that account for the combined negative effects of increased temperatures and acidification on calcification rates, as described for the coralline *Porolithon onkodes* (Anthony et al., 2008; Diaz-Pulido et al., 2012). The information suggests that although temperature softened the detrimental effects of acidification (and increased CO_2) in *L. crispatum* and *S. capensis,* this seems to be an intraspecific feature that cannot be applied to all calcifying macroalgae.

Another feature assessed in *S. capensis* was its phenolic content upon exposure to increased temperatures and decreased pH. Total phenolics increased at nowadays temperatures under acidification, but also at increased temperatures and subject to lower pH. Different investigations have revealed that increased temperatures and acidification are

capable of inducing an oxidative stress condition due to de over-production of ROS by different metabolic pathways (e.g. Celis-Plá et al., 2017; Flores-Molina et al., 2016; Pospíšil, 2016). It has been well described the role of phenolic compounds as antioxidants to counteract ROS-excess (Moenne et al., 2016); thus, the records may indicate that *S. capensis* induces the production of phenolic compounds to inactivate ROS and avoid oxidative damage.

Short-term assessment of temperature and acidification conditions on physiological and metabolic features of *L. crispatum* and *S. capensis* reinforce the importance of local stressors to promote local baseline shifts. In addition, similar records on other macroalgae species suggest that short-term responses may be similar to those expressed in a long-term and, thus, may provide a good representation of potential tolerance and survival thresholds under upcoming environmental conditions. For instance, Chen et al. (2017) observed that within 60 min, light-saturated net photosynthetic O_2 evolution rate (NPRm) in the green macroalga *Ulva lactuca* decreased steadily when the pH dropped from 8.2 to 7.5 and, even more, to 6.5. After this time, NPRm decrease stopped and levels were maintained without a trend of change. Indeed, similar NPRm levels were observed in the alga exposed to pHs 7.5 and 6.5 at 60 min, 90 min and 8 days of experiments. Similar records on short-term exposure (within 48 h) to future climate change scenarios have recorded in other macroalgae species belonging to different phylogenetic groups (e.g. Celis-Pla et al., 2014; Cruces et al., 2017; Flores-Molina et al., 2016).

In conclusion, we observed that both macroalgae species, the coralline *L. crispatum* and the aragonite *S. capensis,* displayed similar physiological responses to acidification. Moreover, and considering the interaction of higher sea surface temperatures and acidification, the information suggests that both assessed macroalgae species may be able to cope and develop under expected future marine environmental scenarios mediated by Climate Change.

Acknowledgements

We thank financial support for Master studies from Becas Chile Magister 2010 (CONICYT, Chile) to Pamela Muñoz. This work was supported by grants from Boticario Foundation; FAPESC—Foundation Support Research and Innovation in the State of Santa Catarina; CNPq—National Council for Scientific and Technological Development; Capes-Higher Education Personnel Improvement Coordination; REBENTOS—Habitat Monitoring Network Coastal Benthic and ProspecMar-Islands— Sustainable Prospecting in Ocean Islands: Biodiversity, Chemistry, Ecology and Biotechnology; Rede Coral Vivo and REDEALGAS; Brazilian Research Network on Global Climate Change, FINEP/Rede CLIMA Grant Number 01.13.0353-00 and FAPESP—Foundation Support Research and Innovation in the State of São Paulo (FAPESP, 2014/00012-1).

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